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TITLE: Cell Cycle Regulation in Prostate Cancer and Its Role in the Transition of Androgen-Dependent Prostate Cancer to Androgen-Independancy

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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) Cell proliferation in the prostate is dependent upon androgen and is associated with specific cell cycle alterations. To examine the relation between cell cycle regulation and androgen-induced proliferation in prostate cancer the transferable human prostate tumor MDA Pca2b was employed. This cell line is androgen-independent but has shown to proliferate in response to androgen. Pca2b tumors were grown in nude mice and when tumors had reached a size of 0.5 cm tumor-bearing animals were castrated. At 3 weeks after castration mice were injected daily with testosterone propionate to replace androgen. Tumor tissue was collected from intact mice, from castrated mice at several time points after castration and from castrated, androgen-treated mice at several time points after start of androgen-replacement. Protein isolated from the tumor samples was subjected to western blot analysis. Cyclins D1, A, and E, cdk 2 and 4, p21 and p27 were present at detectable levels in Pca2b. No significant changes in the levels of these cell cycle proteins were detected upon castration or androgen replacement. It was concluded that the androgen-induced proliferative response of MDA Pca2b is not mediated via the cell cycle pathway.				
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Introduction

Cell proliferation in the prostate is dependent upon androgen. Androgen-induced proliferation of the prostate is associated with specific changes in the cell cycle regulatory proteins. Testosterone has been shown to upregulate expression of certain cyclins and to downregulate cdk inhibitors. The purpose of this research is to study the effect of androgen on cell cycle regulation in the neoplastic prostate. Hereto, the MDA Pca 2b cell line grown as a xenograft in nude mice is used as a model. This cell line was established from a bone metastasis from a patient whose prostate cancer showed androgen-independent growth (Navone et al., 1997). MDA Pca2b was shown to proliferate upon addition of androgen. Tumors were collected from intact mice and at various time points after castration and subsequent androgen replacement of tumor-bearing animals. Tissue was snapfrozen for protein isolation. Protein isolates were subjected to western blot analysis to detect various positive and negative regulators of the G1 phase of the cell cycle.

Body

The Tasks as outlined in the original, approved, Statement of Work were:

- *Task 1.* Determine the effect of androgen on cell cycle regulation in androgen-dependent tumors
- *Task 2.* Transfect androgen-dependent prostate tumor cell lines with wild type p27
- *Task 3.* Analyze androgen-dependent and -independent tumor pairs for AR mutations and expression of cell cycle regulatory genes

Task 1 was completed in the second half of the supported training time. The training and research accomplishments associated with this Task will be discussed in detail below. From the results of Task 1, it was concluded that androgen has no effect on the cell cycle regulation of the androgen-sensitive and androgen-independent prostate cancer cell line Pca2b.

Task 2 aims to prevent the transformation of androgen-dependent prostate cancer cells into androgen-independent cells by transfecting the cells with wild type p27. However, since funding for this training grant has been awarded, it has been shown that p27 is rarely mutated in prostate cancer (Amanatullah et al., 2000). In addition, studies published so far suggest several mechanisms for acquiring androgen independence which all involve the androgen receptor (reviewed by Abate-Shen and Shen, 2000). For these reasons, it appeared no longer worthwhile to pursue Task 2.

Task 3 was to be conducted with the androgen-dependent human prostate cancer xenograft LAPC-9, as stated in the first Annual Summary Report (July 1, 1999-June 30, 2000). It has been reported that 50% of male SCID mice implanted with LAPC-9 form tumors 5 weeks after implantation. The appearance of androgen-independent tumors would take 26 weeks (Craft et al., 1999). Thus, it would take 8 months, though 10 months would probably be more realistic, before pairs of androgen-dependent and -independent prostate tumors would be available. Since only 12 months were remaining of the supported training time, this would leave insufficient time for analysis. It seemed not worthwhile to spend time and resources on the pursuit of Task 3. Furthermore, it turned out that the results reported in the annual summary report for July 1, 1999-June 30, 2000 were based on impure protein isolates. It appeared more beneficial to the trainee to spend the remaining supported training time on developing the trainee's skills in the techniques essential for the studies, i.e. protein isolation and western blotting. Therefore, Task 3 was abandoned in favor of both further development of the trainee's technical skills and completion of Task 1. In addition, the trainee worked on a collaboration project with Dr. John DiGiovanni (see Other training accomplishments) during the remaining supported training time.

Training and research accomplishments associated with Task 1

Training accomplishments

The trainee was taught extensively in protein isolation and western blotting. As a result, the trainee understands the function of each step in these procedures and is able to troubleshoot technical problems. Also, the trainee is capable of adequate evaluation of western blots. The trainee is aware that meaningful analysis of samples in western blotting is only possible if the correct procedures for protein isolation and western blotting

have been followed. For example, protein concentrations of all samples need to be measured on the same day because inherent variations in the protein assay. Likewise, samples from different time points can only be compared if they are run on the same gel.

Furthermore, the trainee received excellent training in tissue culture techniques and learnt about in vitro models of prostate cancer.

Research accomplishments

The trainee isolated protein from all tumor samples. The trainee subjected samples from 9 time points and subjected these together with a molecular weight marker to western blotting and subsequent transfer to nitrocellulose membrane. Several membranes were used to test antibodies. Eventually, the trainee identified antibodies that detected cyclins A, D1, E, cdk2, cdk4, p21 and p27 in Pca2b. Detection of p16 was inconsistent and thus was left out of the analysis. Proteins isolated from tumors of intact, castrated (days 1, 2, 5 and 19 after castration) and castrated mice treated with testosterone propionate (days 1,2,3 and 5 after start of the treatment, which was begun 3 weeks after castration) were analyzed for the presence of cyclins A, D1, E, cdk2, cdk4, p21 and p27. Marginal differences were found between the various samples (see Appendix A). Next, other samples from the same time curve were analyzed by western blotting and, to control for equal loading, strips from the same membranes were probed with actin antibody. From the actin blots, it turned out that samples had been loaded unequally (see Appendix A).. Again, differences in levels of cell cycle proteins between the samples were marginal. Since the differences were far less than 2- to 4-fold, these differences are likely to be due to biological variability, not to an effect of androgen removal or replacement on cell cycle regulation.

Other training accomplishments

During the training period the trainee rewrote a manuscript, entitled “Cell Growth in Response to Androgen in the Mouse Prostate Epithelium” and prepared this for journal submission. The trainee learnt to write clearly and in a logical order.

An ongoing research project at Science Park is the characterization of the K5-IGF1 transgenic mouse, which was developed by Dr. John DiGiovanni and develops spontaneous prostate adenocarcinomas. As part of this project, the trainee dissected ventral prostates of nontransgenic mice and K5-IGF1 transgenic mice of various ages and stained these to detect keratin 5 –a marker of basal cells- and, as a measure of proliferation, bromodeoxyuridine. The trainee perfected her skills in dissecting and processing of mouse ventral prostates, techniques which allow the study of the spatial relationships between prostate epithelial cells.

During the second half of the supported training time the Army grant, which the trainee co-wrote during the first half of the supported training time, was approved for funding. The trainee provided the information requested by the funding organization for finalizing the funding and, as a result, learnt more about the grant writing process.

The trainee learnt how to use the internet technology for transfer of scientific information. This training is considered valuable since information transfer via the world wide web is essential for scientific research. The trainee created two web sites which are based on knowledge gained during the supported training time. One web site discusses the dissection of the mouse ventral prostate (<http://www.ccsi.com/~frijhoff/intro.html>). The second web site gives information about basic histology procedures and the services provided by the Science Park Histology Core Facility (<http://www.ccsi.com/~frijhoff/WebHelp/frijhoff4a.htm>). These web sites will be useful for researchers at Science Park.

Key Research Accomplishments

- Protein purification from tumor samples.
- Detection of human cyclin D1, A, E; cdk 2, 4; p21, p27 by western blotting
- Western blotting of samples of all time points for cyclin D1, A, E; cdk 2, 4; p21, p27. These blots showed unequivocally that androgen removal or replacement does not affect Pca2b at the level of cell cycle proteins.

Reportable Outcomes

Manuscript, entitled "Cell Growth in Response to Androgen in the Mouse Prostate Epithelium", which will be submitted to *Cell Growth and Differentiation*.

Conclusions

Training

During the supported training time the trainee has gained insights into scientific research. Discussions with the mentor and colleagues have made the trainee aware of the various approaches to scientific questions. Also, the trainee has come to understand how technical difficulties can influence the analysis of samples. The training was very successful in making the trainee knowledgeable about prostate cancer. The trainee learned about the pathology of mouse prostate. At the intellectual level, the trainee gained a better understanding of the problems associated with the different prostate cancer models and of the areas of importance in prostate cancer research. While the trainee will not pursue a career in prostate cancer research, the support by the DAMD award has given the trainee a solid training in scientific research. This will allow the trainee to inform adequately about science in her future career as technical writer.

Research

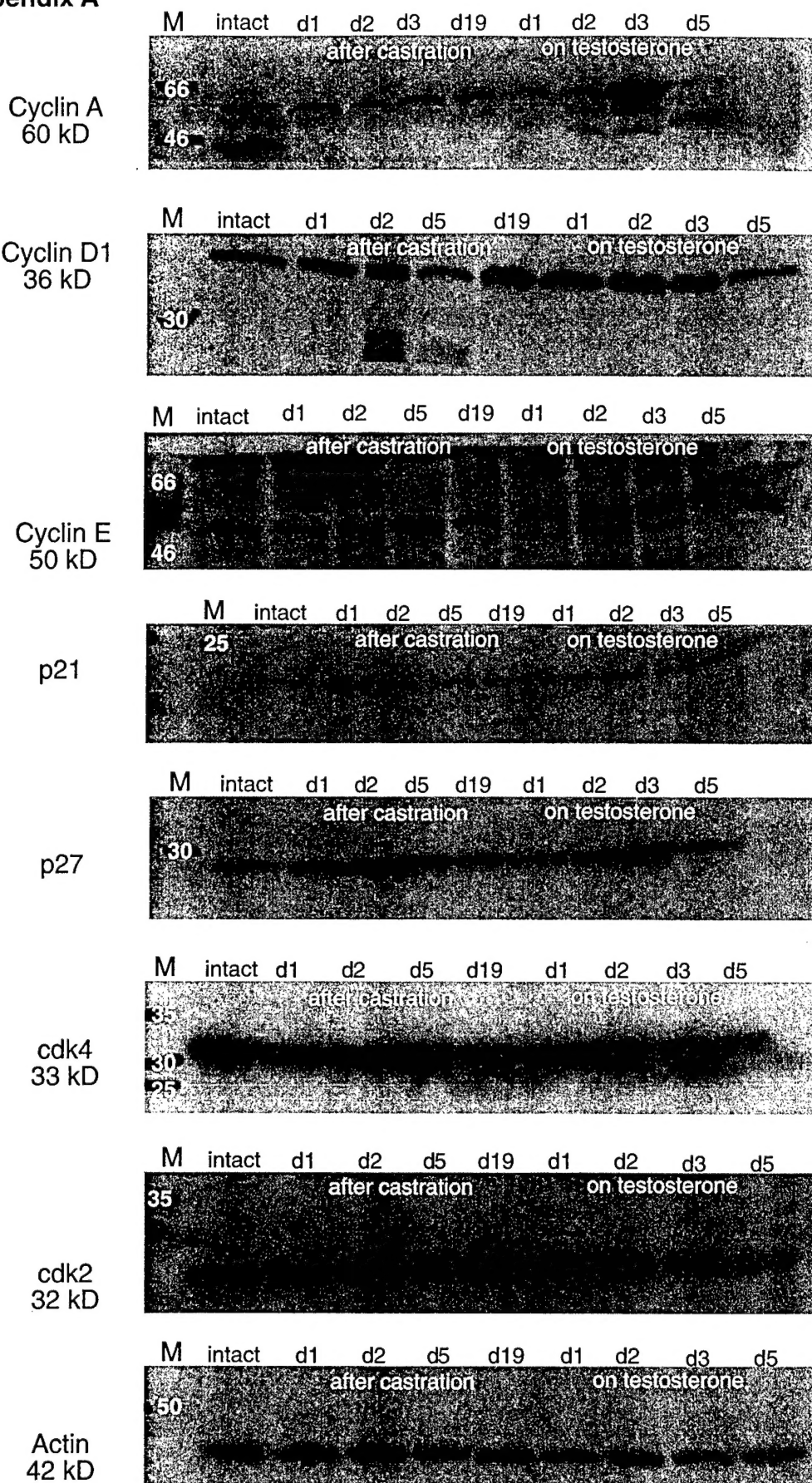
Androgen has been shown to induce proliferation of Pca2b (Navone et al., 1997). The results obtained during the supported training time showed that levels of cell cycle proteins in Pca2b are not affected by the presence or absence of androgen. The effect of androgen on Pca2b cell proliferation may be mediated via a signal transduction mechanism. A human prostate xenograft was shown to acquire androgen-independency when, following transfection, the tumor cells over-expressed the HER-2/neu receptor tyrosine kinase (Craft et al., 1999).

While androgen has no effect on the cell cycle regulation in Pca2b, it is well-known that prostate cancer is a heterogeneous disease (Abate-Shen and Shen, 2000). Thus, the cell cycle in other androgen-responsive/ androgen-independent prostate xenografts may be regulated –to various extents- by androgen.

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Appendix A



Appendix A. MDA Pca 2b tumors were collected from intact mice, castrated mice (at days 1, 2, 5 and 19 after castration), and castrated mice which were treated with testosterone propionate(1, 2, 3 and 5 days after start of the treatment). Protein was isolated from the tumors and analyzed by western blot for the presence of the indicated cell cycle proteins. M, molecular weight marker.